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Research Article

CARDIO PROTECTIVE EFFECT OF THE LEAVES OF *Artocarpus heterophyllus* L. ON *Daphnia magna*

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ABSTRACT

Objective: To prescreen the *in vivo* cardioprotective activity of the leaves of *Artocarpus heterophyllus*.L Family Moraceae using the model organism *Daphnia magna* along with preliminary phytochemical study and acute toxicity assessment.

Method: To evaluate the Cardioprotective effect of the ethyl acetate extract of the leaves of *A.heterophyllus* (EAAH) *in vivo* on the lactose induced arrhythmic heart of the cladocerans *D.magna* (Water flea) a novel model system for studying effects of agonists and toxins on cell signalling and ion channels in situ. Initially acute toxicity assessment, total phenolic content by UV spectral methods and ursolic acid content by HPTLC, trace elements by X-ray fluorescence were determined.

Results: Normal mean heart beat of the *D.magna* at 20^0±2°C was found to be 191.4±1.4 beats/min (n=50). Arrhythmia was induced by lactose (200mM) in the bathing medium. The ethyl acetate extract of the leaves of *A.heterophyllus* (20, 40, 60, 80µg/ml) prevented the lactose induced arrhythmia in dose dependent manner. Previous assessment of toxicity showed LC_{50} 5.88mg/L. Preliminary phytochemical screening of appropriate solvent extract of the leaves showed the presence of flavonoids, sterols, carbohydrates, proteins, tannins , phenolic compounds and absence of alkaloids, volatile oils, fixed oils, glycosides like anthroquinone, cardiac, cyanogenetic and isothiocyanate. Total phenolic, ursolic acid content of EAAH was 376.5mg/g, 134mg/g respectively. High percentage of calcium, potassium was found in addition to traces of magnesium, sulphur, zinc, strontium, manganese, aluminium.

Conclusion: *Artocarpus heterophyllus* L. (Jack fruit) has long been recognized and economically is of appreciable importance as a source of edible aggregate fruit. This study indicates that the ethyl acetate extract of the leaves of *A.heterophyllus* possesses potential cardio protective activity on the lactose induced arrhythmia of the *Daphnia* heart without any toxicity and mortality. It is assumed that this may be due to polyphenolic content, ursolic acid, trace elements like calcium, potassium, magnesium. Further investigation requires confirming this activity.

Keywords: *Artocarpus heterophyllus*, Moraceae, Cardioprotective, *Daphnia magna*, Ursolicacid

INTRODUCTION

Artocarpus genus (Family: Moraceae-mulberry family) received a great level of scientific interest as they consists of therapeutically active secondary metabolites and is economic source of food and widely used in traditional medicine. *Artocarpus* species are used as food and for traditional folk medicine in South-East Asia, Indonesia, Western part of Java and India. *Artocarpus* plants offer advantages as a profitable multipurpose crop for producing fruits and timber. *Artocarpus* has long been recognised and economically is of appreciable importance as a source of edible aggregate fruit; such as *Artocarpus heterophyllus* (Jack fruit), *Artocarpus altilis*(bread fruit) and *Artocarpus chempeden*(Chempedak) and yielding fairly good timber[1]. *Artocarpus heterophyllus* popularly known as jack fruit is one of the important and commonly found trees in the home gardens of India and Bangladesh. The term jackfruit is derived from the Portuguese word Jaca which in turn is adopted from the word "Chakka" of Malayalam - A regional Indian language [2]. Extracts of its plant parts have been applied in traditional medicine for the treatment of diarrhoea, diabetes, malarial fever, tapeworm infestation, and as wound healing, antisyphilic, expectorant and also to treat anaemia, asthma and dermatitis [1]. The present study investigate the cardiac effect of the ethyl acetate extract of the leaves of *A.heterophyllus* (containing polyphenols including flavonoids with triterpenoid ursolic acid) using the model organism *Daphnia magna*. The small fresh water crustacean *D.magna* (0.2-3mm) was used in this experiment because of their transparent carapace, which allows for increased visibility of the internal organs and makes monitoring the heart rate of the individual easier [3]. Of all sequenced genomes belonging to the animal group composed of insects and crustaceans, *Daphnia* share more genes with humans [4]. It exhibits a short life span, rapid maturation and reproduction. The heart of the water flea, *D.magna*, regulated by cholinergic neurons and may be useful as a model for the effect of drugs on cardiovascular function and unusual among crustaceans in that they possess myogenic hearts. Testing the effects of the drugs is simplified in *D.magna* as the fleas are responsive to pharmacological agents added to the water in which they swim. The introduction of these pharmacological agents to water fleas may induce activity directly on the cardiac muscle [5].

MATERIALS&METHODS

Lactose, Elendt and Bios medium, spirulina, ethylacetate. All chemicals used are Sd fine chemicals. For the determination of trace element by X-ray flourescence Bruker S-4 pioneer and CAMAG HPTLC with winCATS 1.4.3 software, densitometry TLC scanner (520nm) was used for HPTLC analysis. Laboscope model Microscope with Photomicrograph & CCTV.

Collection and authentication of the leaves of *A.heterophyllus*: The leaves of the healthy *A.heterophyllus* selected for our study was collected from Susendram, Kanyakumari (Dt), Tamilnadu. It was identified and authenticated by Prof. Dr.P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamilnadu, India and Dr.Stephen, Taxonomist, Dept. of Botany, The American College, Madurai. A voucher specimen was deposited at the herbarium of Dept. of Pharmacognosy, Madurai Medical College, Madurai, Tamilnadu, India (PCG-276).

Preparation of extract: The leaves were dried at room temperature under shade and powdered, sieved (60mesh) and stored in a well closed container. Extracted with ethylacetate and filtered, evaporated under vacuum (Rotavapor RII, Buchi). The pale green residue obtained (EAAH) was stored in the refrigerator until further use.

Preliminary phytochemical screening: Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [6, 7]

Determination of Total Phenolic Content: The total phenolic content of extracts was determined by Folin-Ciocalteau method [8]. The extracts were oxidized with Folin-Ciocalteau reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting solution was measured at 760 nm after 20min. Using gallic acid as standard total phenolic content (standard curve was prepared using concentrations 25-50 mg/L) was expressed as mg GA equivalent/L of extract.

Elemental analysis by XRF Spectrometer: We have quantitatively determined the trace elements present in the *A.heterophyllus* leaves by X-Ray flouresence spectrometer (XRF) which has the advantage generally being non-destructive, multi elemental, fast & cost effective [9, 10].

Preparation of solid sample:

Mix equal volume of powder and binder pressed up to 30 ton made into pellet. The binder must be free from contaminant element and low absorption. It must stable under vacuum and irradiation conditions.

HPTLC profile of EAAH:

Development of HPTLC fingerprint

Instrument

CAMAG TLC Scanner 3 "Scanner3-070408"S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software used was Win CATS 1.4.3

Sample

The EAAH was dissolved in ethyl acetate to get a concentration of 2mg/ml and 2µl of this solution was used for taking HPTLC fingerprint.

Stationary Phase

Aluminium sheets pre-coated with silica gel Merck G F_{254}, 0.2mm layer thickness were used as the stationary phase.

Mobile phase

Toluene: Ethyl acetate: Methanol (7:2:1) was used as the mobile phase for development of chromatogram. The mobile phase was taken in a CAMAG twin trough glass chamber.

Detection wavelength

The developed plates were examined at wavelength 520nm in Densitometry TLC scanner 3. The TLC visualization, 3D display of the finger print profile and peak display at 520nm.

Culture of *Daphnia magna*: *D.magna* obtained from the local aquarium in Madurai, Tamilnadu, India. It was identified & authenticated by Prof (Major) P.Chandrasekaran, Principal, Manonmaniam Sundaranar University Constituent Model College,Vilathikulam, Nagalapuram 628 904, Thoothukudi Dt,Tamil Nadu, India. (Formerly Faculty of PG and Research, Dept of Zoology and Biotechnology, Vivekananda College, Thiruvedakam West 625 217, Madurai, Tamilnadu, India. *D.magna* were cultured by using Elendt-Bias(M_4) medium[11] and maintained photoperiod ±12hr. Spirulina used as a feed in spring water aerated for 48hr to obtain O_2 concentration not less than 4mg/ml. Experiment was carried at 20°C±2°C.

Toxicity assessment of *A.heterophyllus* leaf: 48 hr exposure of *D.magna* to different concentrations (1, 2, 3, 4, 5, 6 mg/L) of EAAH was observed. One day old *daphnids* were selected for this study, since neonates may be more susceptible to toxic substance than elder one. Moreover more specificity, simplicity including easily handle in lab & less expensive. Temperature 20°C ±2°C is maintained. No food feed through the study. Test substance was added directly to the water at various concentrations. Mortality rate was observed after 24 hr and LC_{50} was determined [12].

in vivo* Cardioprotective activities of the EAAH leaves on *Daphnia magna: The heart rate of control & treated groups (Lactose and EAAH 20, 40, 60 and 80µg/ml) were monitored by transferring *D.magna* to depression slide slightly coated with petroleum jelly [4].

Heart beats were observed under light microscope and recorded by using Nikon coolpix camera. It was counted by image processing technique which allowed real time operations i.e. 25frames/sec.

RESULT

1. Preliminary phytochemical screening of EAAH leaves showed the presence of flavonoids, sterols, carbohydrates, proteins, tannins, phenolic compounds and absence of alkaloids, volatile & fixed oils, glycosides like anthroquinone, cardiac, cyanogenetic and isothiocyanate.
2. The total phenolic content of EAAH was found to be 376.5mg/g.
3. Trace element content by XRF analysis showed the presence of calcium (39.4%), potassium (29.6%), magnesium (2.06%), Iron (0.99%), sulphur (1.83%), zinc (0.083%), strontium (0.23%), manganese (0.13%) and aluminium (0.005%).
4. HPTLC analysis of EAAH contains 134mg/g of ursolic acid (Figure 1&2, Table 1).
5. From the result of toxicity assessment of EAAH it is clearly observed that EAAH is safe and non toxic to *D.magna*. No significant mortality was observed up to 6mg/L. LC_{50} 5.88mg/L (Fig 3).
6. The heartbeat of control, Lactose induced, EAAH 20, 40, 60, 80 µg/ml treated were found to be 191.4±1.4, 92±1.29, 139.9±1.27, 160.6±0.88, 189.4±0.74, 190.1±0.54bpm respectively (Fig 4).

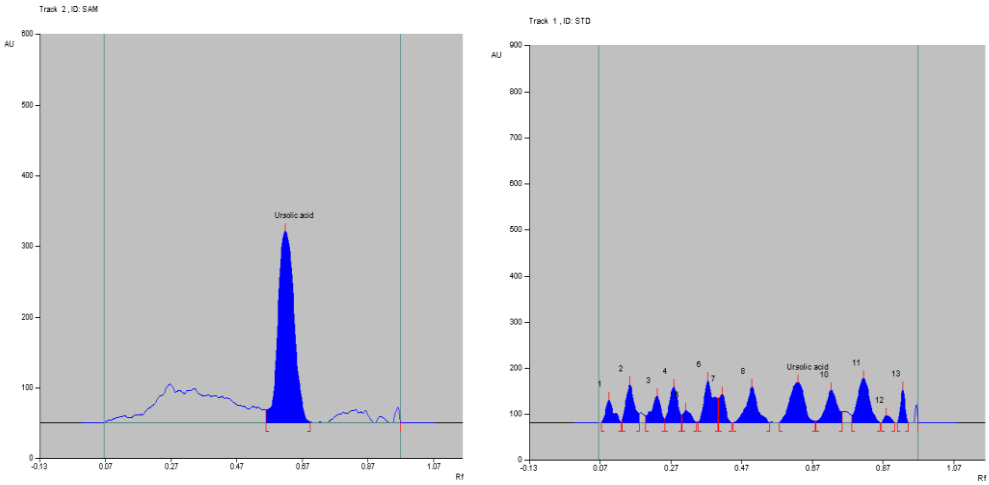

Figure-1 HPTLC peak display

Table-1: R_f value and area of separated compounds

S. No	@520nm			
	R_fValue		AREA (AU)	
	TRACK			
	STD	Extract	STD	Extract
1		0.10		781.2
2		0.15		1369.7
3		0.23		1063.7
4		0.28		1286.5
5		0.31		428.3
6		0.38		1813.0
7		0.42		801.7
		0.50		1748.4
	0.62	**0.63**	**9451.7**	**2534.1**
		0.72		1649.0
		0.82		2308.4
		0.88		211.7
		0.93		612.9

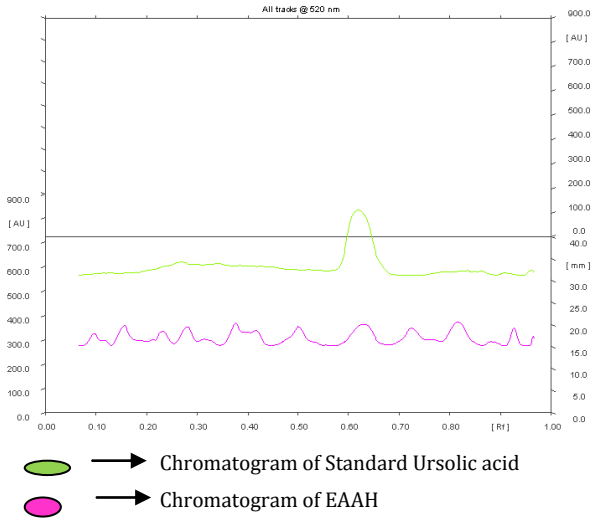

Figure- 2: CO-HPTLC profile of EAAH showing the presence of Ursolic acid 3D display

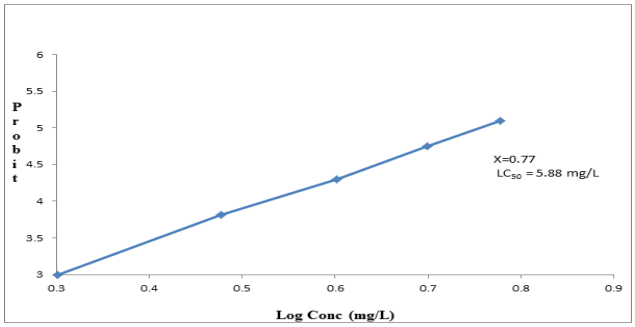

Figure-3: LC_{50} OF EAAH Leaves

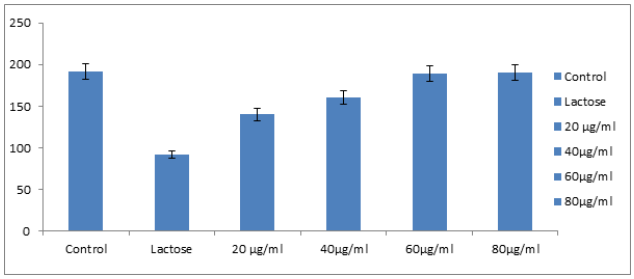

Figure-4: heart rate of control and different concentrations of EAAH treated on the lactose induced arrhythmic heart of *D.magna*

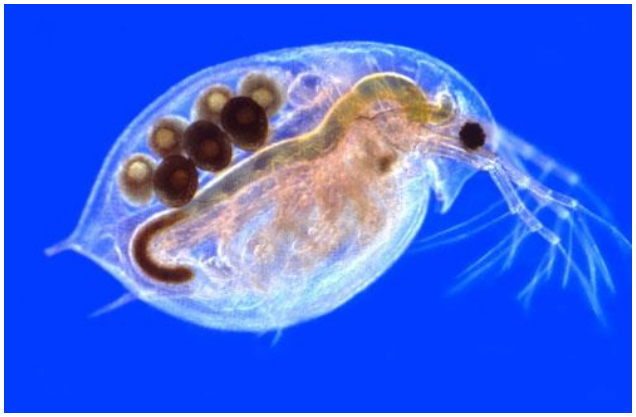

Image of *Daphnia magna* [Water flea]

DISCUSSION

Antibacterial activity, anti cariogenic, anti cancer, hypoglycaemic, α-amylase inhibitory, wound healing, anti asthmatic, anti syphilic, vermifuge, lactogogue, analgesic, anti ulcer activities of leaves of *A.heterophyllus* have been reported[13-22]. In recent years, phytochemical constituents of plants with varied pharmacological, physiological and biochemical activities have received attention. Plants rich in bioactive phytochemical reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular and oxidative dysfunction. A great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties. Studies have shown that *A.heterophyllus* contains many classes of compounds such as flavonoids, volatile acids, sterols and tannins. It was also reported that whole aqueous extract of the leaf observed to possess the hightest phenolic content (523.2mg/g) Leaves contains prenyl flavones (artocarpine, artocarpetin, artocarpetin A, cycloheterophyllin and artonins A and B) with antioxidant activity [2]. In our study it was observed that EAAH contains 376.5mg/g phenolic content. Most of the pharmacological effects can be explained by the phenolic compounds including flavonoids, stilbenoids, aryl benzofurans present in all parts of the plant [23]. It was reported that root of *A.heterophyllus* contains beta sitosterol, ursolic acid, betulinic acid, cycloartenone and artoflavanone [24]. Ursolic acid and oleanolic acid are pentacyclic triterpenoids that are present in many medicinal herbs and other plants. It was reported that they are anti-inflammatory, hepatoprotective, analgesic, cardiotonic, antihyperlipidemic, sedative [25]. It prompted us to find out the presence of ursolic acid in the leaf of *A.heterophyllus*. It was found out by HPTLC that EAAH contains 134 mg/g of ursolic acid. Based on the above facts we have investigated the effect of EAAH on the lactose induced arrthymia of *D.magna* heart. The results clearly showed the dose dependent protective effect on lactose induced arrthymia of the heart of *D.magna* by EAAH (Fig.2). It is assumed that this cardioprotective effect may be due to the phenolic content, ursolic acid and the influence of abundant calcium, potassium, magnesium content and antioxidant activity. Assessment of acute toxicity study reveals its safety and non-toxic nature. So it is concluded that leaves of *A.heterophyllus* possesses cardio protective effect without toxicity. Further investigation on animal model and clinical trials are required.

Conflict of interest statement

We do not have any conflict of interest.

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